VIROLOGY 63, 263-272 (1975)

A Transcriptional Map of the SV40 Genome in Transformed Cell Lines

GEORGE KHOURY AND MALCOLM A. MARTIN

Laboratory of Biology of Viruses, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014

THERESA N. H. LEE AND DANIEL NATHANS

Department of Microbiology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Accepted September 5, 1974

An analysis of viral DNA transcription in several SV40 transformed cell lines indicated the presence of both abundant and scarce classes of viral specific RNA. The topographical location of the SV40 DNA sequences which were complementary to transformed cell RNA was determined in hybridization experiments utilizing the separated strands of the 11 SV40 DNA fragments produced by digestion with the *Hemophilus influenzae* (R·*Hind*) restriction endonuclease.

Extensive hybridization of the transformed cell RNA with the minus DNA strands of *Hin* fragments A, H, I and B indicated that the abundant species of viral-specific RNA was similar, if not identical, to early SV40 RNA. The less abundant class of viral RNA was complementary to regions of the minus DNA strand contiguous with the early region, particularly those sequences located at the 3'-DNA end of this segment of the genome. Viral-specific RNA, complementary to the plus DNA strand and present in low concentrations in only a few transformed cell lines, could not be localized on the genome.

INTRODUCTION

Transcription of SV40 DNA during productive infection of monkey kidney cells has been shown to occur in two phases. Prior to viral DNA replication, early RNA is transcribed from approximately one-third of the minus DNA strand. Late in the lytic cycle, the stable species of viral-specific RNA has been shown to be complementary to one-third of the minus strand and approximately two-thirds of the plus strand (Lindstrom and Dulbecco, 1972; Khoury *et al.*, 1972; Sambrook *et al.*, 1972). The mapping of transcription sites expressed in productively infected cells (Khoury *et al.*, 1973b; Sambrook *et al.*, 1973) was determined using the separated strands of restriction enzyme fragments of the viral genome. These results indicated that stable early RNA was transcribed from a continuous segment of the SV40 genome (31% of the DNA) and that stable, late SV40 transcripts were transcribed from a continuous segment (about 69% of the DNA). Recent studies using cytoplasmic RNA prepared from infected cells late in the SV40 lytic cycle indicate, however, that the early SV40 region encompasses most, if not all, of the minus strand DNA sequences corresponding to *Hin* fragments A, H, I, and B (approximately 47% of the genome; see Fig. 3; Khoury *et al.*, manuscript in preparation). This observation and the finding of low, yet significant levels of reaction of total cellular late RNA with regions of the minus DNA strand (Khoury *et al.*, 1973b) suggested that the expression of viral genetic information was a complex process. The initial RNA product thus appears to be complementary to extensive regions of both DNA strands, as proposed by Aloni (1972).

The pattern of transcription of SV40-transformed cell lines is clearly different from that observed in lytically infected cells (Khoury *et al.*, 1973; Ozanne *et al.*, 1973; Martin and Khoury, 1973). In the 11 transformed lines examined in this laboratory, varying portions of the minus (early) viral DNA strand were expressed; very low levels of reaction were observed between SV40-specific RNA from only three transformed cell lines and the plus (or late) DNA strand. The stable species of viral-specific RNA present in transformed cells includes the RNA species transcribed early in the productive cycle (Aloni *et al.*, 1968; Oda and Dulbecco, 1968; Sauer and Kidwai, 1968; Khoury *et al.*, 1973a) and additional RNA derived from other regions of the minus DNA strand (referred to as "anti-late" RNA) (Sambrook *et al.*, 1972; Khoury *et al.*, 1973a).

In the present study we have extended the prior analysis of transcription of SV40 genes present in transformed cells by mapping the stable SV40 RNA found in several transformed cell lines. For this purpose we have reacted RNA from transformed cells with separated strands of individual endo R·*Hin*d (*Hin*) fragments of SV40 DNA (Danna and Nathans, 1971, 1972; Danna *et al.*, 1973). Since the order of these fragments in the viral DNA molecule is known, we could determine what regions of the SV40 genome are transcribed in transformed cells and relate these patterns of transcription to that found in lytically infected cells.

MATERIALS AND METHODS

Virus and viral DNA. Confluent monolayers of the BSC-1 line of African green monkey kidney cells were innoculated with plaque-purified SV40 (small plaque, from strain 776) at a multiplicity of 0.01 PFU/cell. Carrier-free [^{32}P]orthophosphate was added 24 hr after infection; the ^{32}P-labeled SV40 DNA I was prepared from purified virus by equilibrium density centrifugation in CsCl-ethidium bromide, as previously described (Khoury *et al.*, 1972). The specific activity of the DNA preparations ranged from 1.5–6.0 × 10^5 cpm/μg. Endo R·*Hin*d fragments of SV40 DNA were prepared as previously described (Danna and Nathans, 1971) and the individual strands separated by reaction with cRNA (Khoury *et al.*, 1973b). SV40 cRNA was made from supercoiled SV40 DNA with *Escherichia coli* DNA-dependent RNA polymerase (kindly provided by Dr. T. Shih).

Transformed cell RNA. Total cellular RNA from SV40 transformed cell lines was prepared from confluent monolayers by the hot phenol method as previously described (Khoury *et al.*, 1973a). SV-3T3 cellular RNA was kindly provided by Dr. Dona Lindstrom. It was prepared by the method of Penman (1966).

Size of separated SV40 DNA strands. The size of the separated strands of three representative ^{32}P-labeled SV40 DNA fragments (produced by cleavage with R·*Hin*d restriction endonuclease) was determined by sedimentation in alkaline sucrose. Samples of 0.1 ml containing 10–20 μg of a separated strand of SV40 *Hin* fragment A, D or G in 0.14 *M* phosphate buffer were layered on the surface of a 12 ml 10–30% alkaline sucrose gradient (0.7 *M* NaCl, 0.3 *M* NaOH, 0.0025 *M* EDTA and 0.015% Sarkosyl) and sedimented at 10° in an SW41 rotor at 40,000 rpm for 36 hr. At the end of the centrifugation period 0.24 ml samples were collected dropwise from the centrifuge tube directly into scintillation vials. Three drops of glacial acetic acid and 10 ml of an aqueous scintillation fluid were added to each vial. The samples were then counted in a Beckman LS256 liquid scintillation counter.

DNA-RNA hybridization. Small amounts (1–5 ng) of the plus or minus strand of SV40 DNA fragments (*Hin* A–K) were incubated with large amounts (0.1–8.0 mg/ml) of transformed cell RNA in the presence of 1.0 *M* NaCl, 0.04 *M* phosphate buffer (PB), pH 6.8, 0.002 *M* EDTA, pH 7.5, and 0.01% sodium dodecyl sulfate (SDS) in 0.15–0.25 ml, at 68° for 24–30 hr.

RESULTS

Size of the Separated Strands of Hin Fragments

In this study, we have employed hydrox-

yapatite (HA) chromatography to determine the extent of hybridization between transformed cell RNA's and the separated strands of SV40 *H. influenzae* restriction enzyme fragments. Unlike assays that depend on resistance to single-strand-specific nucleases, this method does not distinquish between partial and complete duplex molecules. As the size of the radiolabeled DNA probe becomes smaller, however, the hybridization values obtained with HA approach those observed with specific nucleases. The SV40 *Hin* DNA fragments vary more than fivefold in size (Danna and Nathans, 1971). Degradation of these fragments during their isolation and subsequent strand separation could result in a heterogeneous collection of different sized molecules and make interpretation of the HA results more difficult. It was, therefore, important to determine the size of the ^{32}P-labeled strands of the SV40 *Hin* fragments after strand separation.

The size of separated strands of three *Hin* fragments (A, D, G) was compared to the size of the strands of the same intact fragments by alkaline sucrose sedimentation. Ths results shown in Fig. 1 indicate that some fragmentation occurs during the strand separation procedure, primarily with the larger DNA fragments (e.g., *Hin* A). In fact, there appears to be a relative limit in the size to which the nucleotide chains are broken during strand separation (approximately 5–10% of the SV40 unit length). A large *Hin* fragment such as *Hin* A is broken into an average of four segments while the smaller fragments (G–K), for the most part, remain intact. Thus, while the overestimate of the per cent of nucleotide sequences in duplex molecules may be relatively small for a large *Hin* fragment (such as *Hin* A), it is proportionately higher for the smaller *Hin* fragments that remain intact.

Hybridization of Transformed Cell RNA to Individual Strands of Endo R·Hind Fragments of SV40 DNA

In a prior study (Khoury *et al.*, 1973a) it was shown that RNA extracted from a series of SV40-transformed cell lines hybridized extensively with the minus or early template strand (Table 1). In all cases, transformed cell RNA included sequences corresponding to early SV40 RNA found in lytically infected cells (Khoury *et al.*, 1973a), and most lines had additional RNA transcribed from other regions of the minus strand, the so-called "anti-late" transcripts. On the basis of the extent and kinetics of hybridization between transformed cell RNA and SV40 DNA at increasing RNA concentrations, it also appeared that transcripts from different regions of SV40 DNA varied in abundance (Khoury *et al.*, 1973a).

In order to confirm the presence of early SV40 RNA in SV40-transformed cells and, in particular, to map the DNA sites which are complementary to "anti-late" SV40 RNA, we hybridized RNA from transformed cells with separated strands of endo R·*Hin*d fragments of SV40 DNA. Since the order of these fragments in the genome is known (Danna and Nathans, 1972, 1973) and the early and late regions have been mapped (Khoury *et al.*, 1973b; Sambrook *et al.*, 1973) we could relate the results with transformed cell RNA to the lytic transcription map (see Fig. 3A). The results are presented in Table 2.

As shown in Table 2, extensive hybridization occurred between RNA from each SV40-transformed cell line and the minus strands of fragments A, H, I and B. At the RNA concentrations used, the minus strands of fragments A and B were almost fully saturated (>90%) as assayed by hydroxyapatite chromatography. The reactions with the minus strands of *Hin* fragments H and I were also extensive. This reaction of transformed cell RNA with the minus strand of contiguous *Hin* fragments A, B, H and I is similar to that observed with early lytic RNA (Khoury *et al.*, 1973b) and confirms prior findings that the early region of the SV40 genome is transcribed in all of the transformed cell lines studied (Khoury *et al.*, 1973a). The results also indicate that early SV40 RNA is the dominant SV40 species present.

Further analysis of the data in Table 2 and the kinetics of hybridization (Fig. 2), indicates that there is an intermediate

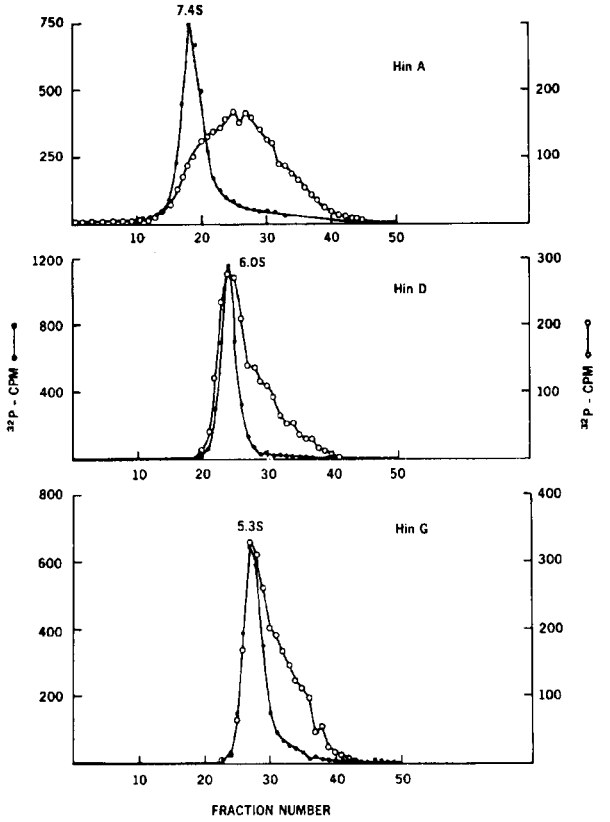

FIG. 1. Determination of the size of the separated strands of three representative *Hin* fragments of SV40 DNA. To determine the effect of strand separation procedure on the size of [^{32}P]SV40 DNA *Hin* fragments, alkaline sucrose sedimentation studies were performed on *Hin* fragments A, D and G, both before (●——●) and after (O——O) strand separation as described in Materials and Methods. Each of the six samples was sedimented in a separate tube in the same experiment. The plus strands were used for the separated strand in this analysis, but similar results were obtained in other studies with minus DNA strands.

level of hybridization between most of the transformed cell RNA's and the minus strand of fragment *Hin* C and between three of the transformed cell RNA's (SV-3T3, SV-S-AL/N, SV-OM/N) and *Hin* D. A much lower level of reaction was found between the RNA from several transformed lines and the minus strand of fragment *Hin* G.

Hin fragments C and D are physically located adjacent to the early region of the SV40 genome (Fig. 3A). Since their reaction with transformed cell RNA was incomplete under conditions where *Hin* fragments A and B were easily saturated (Table 2, Fig. 2), it seems likely that the viral-specific RNA which hybridized with these fragments represents a less abundant or scarce component of SV40 specific RNA in transformed cells.

In earlier studies (Khoury *et al.*, 1973a; Martin and Khoury, 1973), we were able to detect low levels of RNA homologous to the plus (late) RNA strand in only three of 12 SV40 transformed cell lines. The RNA from two of these three lines (SV-S-AL/N and SV-3T3) was incubated with the plus DNA strands of the 11 SV40 *Hin* fragments (Table 2). In neither case were we able to localize these RNA species to specific regions of the plus DNA strand.

Estimation of the Portion of the Minus Strand of Fragments A and B That Hybridize with Transformed Cell RNA's

The reaction of each of the transformed cellular RNA's and the minus strands of fragments A and B was very nearly complete as measured by HA chromatography. Since this method fails to distinguish between partial and complete hybrid molecules and since the separated strands of fragments *Hin* A and B are broken to only a limited extent, the single-strand specific nuclease S_1 was employed to determine the proportion of the minus strands of these fragments complementary to the RNA present in SV40-transformed cell lines as has previously been determined for productively infected cells (Khoury *et al.*, 1973b). The results in Table 3 indicate that between 70 and 90% of the minus strand of both *Hin* fragments A and B are resistant to the nuclease after hybridization with the indicated amounts of different transformed cell RNA's. It should be noted that the extent of hybridization of the minus strands of fragments *Hin* A and *Hin* B is greater with each of the transformed cell RNA's than it appeared to be with total cellular late lytic RNA (Table 3). Nevertheless, more recent studies with late lytic SV40 *cytoplasmic* RNA indicate that most, if not all, of fragments *Hin* A and B are contained in the early SV40 template (Fig. 3A, Table 3; Khoury *et al.*, manuscript in

TABLE 1

HYBRIDIZATION OF SV40 TRANSFORMED CELL RNA TO THE SEPARATED STRAND FRAGMENTS OF SV40 DNA[a]

RNA annealed	Analysis of hybrid molecules		
	HA (% duplex molecules)		S_1 nuclease (% resistance)
	(+) Strand DNA	(−) Strand DNA	(−) Strand DNA
None	<1	2	1
SV40 early lytic (total cell)	<1	46	38
SV40 cRNA	2	99	96
SV-S-AL/N	4	53	42
SV-3T3	9	52	ND[b]
SV-L-AL/N	1	75	59
WI-18-VaZ	<1	77	55
SV-S-OM/N	2	50	39
SV-UV-15 C1 1	<1	48	23
11-A-8	1	50	30

[a] Data from Khoury *et al.* (1973a) and Martin and Khoury (1973).

[b] ND = not done.

TABLE 2

HYBRIDIZATION OF TRANSFORMED CELL RNA WITH THE SEPARATED STRANDS OF ^{32}P-LABELED *Hin* FRAGMENTS[a]

Transformed cell RNA	RNA concentrations (mg/ml)	% Minus strand of *Hin* fragments hybridized										
		A	B	C	D	E	F	G	H	I	J	K
SV-L-AL/N	6.8	93	83	16	10	12	4	8	37	16	2	8
SV-3T3	3.2	92	86	44	24	16	6	10	85	60	7	9
SV-S-AL/N	4.8	88	92	30	29	9	9	9	80	60	3	9
WI-18-Va2	6.0	92	76	22	5	7	5	11	89	83	8	2
SV-S-OM/N	3.2	90	80	27	21	2	0	4	60	49	2	9
SV-UV-15 C1 1	1.5	88	92	17	2	6	6	7	61	24	2	1
11-A-8	3.7	90	89	37	6	3	5	7	75	70	4	1
		% Plus strand of *Hin* fragments hybridized										
		A	B	C	D	E	F	G	H	I	J	K
SV-S-AL/N	6.8	3	0	3	2	6	5	12	0	3	4	4
SV-3T3	3.2	8	4	11	6	6	3	5	5	4	3	7

[a] Per cent [^{32}P]DNA present in duplex molecules after hybridization of transformed cell RNA's with the minus or plus strand of *Hin* DNA fragments A–K as analyzed by HA chromatography (see Materials and Methods for reaction conditions). Background levels of hybridization (<3%) in the absence of RNA were not subtracted from these results.

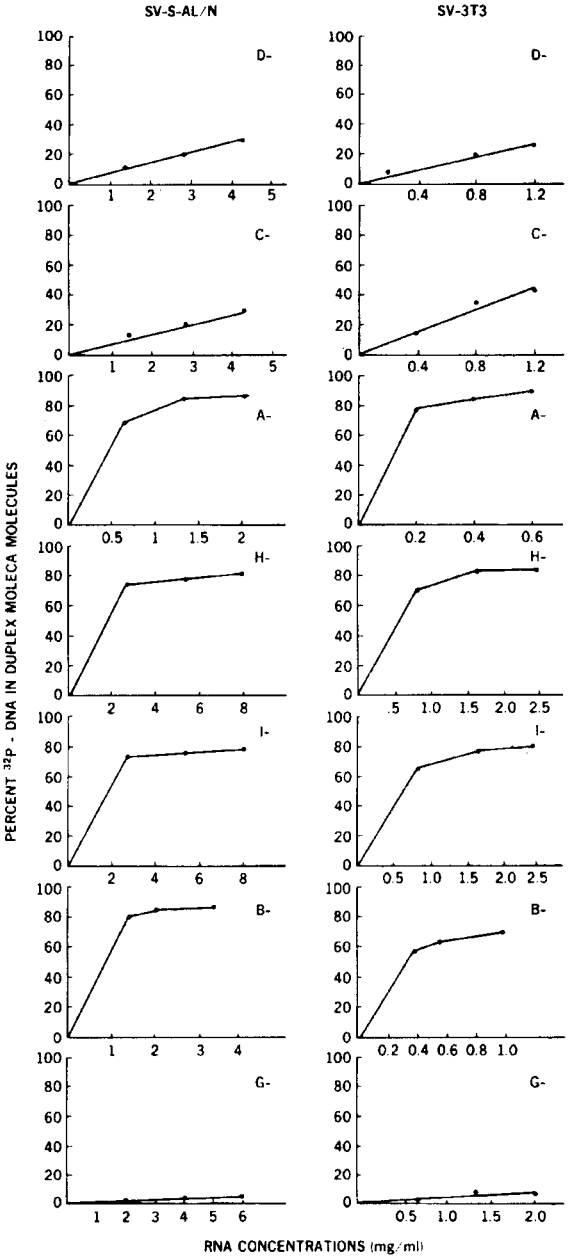

FIG. 2. Kinetics of hybridization between the minus strands of [^{32}P]DNA *Hin* fragments and RNA from two SV40 transformed cell lines. Increasing concentrations of total cellular RNA from either SV-S-AL/N cells or SV-3T3 cells was annealed with approximately 0.5–1.0 ng of the minus strand of [^{32}P]SV40 fragments under conditions described in Materials and Methods. At 36 hrs., the percent of [^{32}P]DNA in duplex molecules was assayed by HA chromatography. Since similar amounts of DNA were used in each reaction, a larger number of copies of the smaller *Hin* fragments than the larger fragments were present. Thus, it should be noted that the concentrations of RNA employed are inversely proportional to the size of the *Hin* DNA fragment.

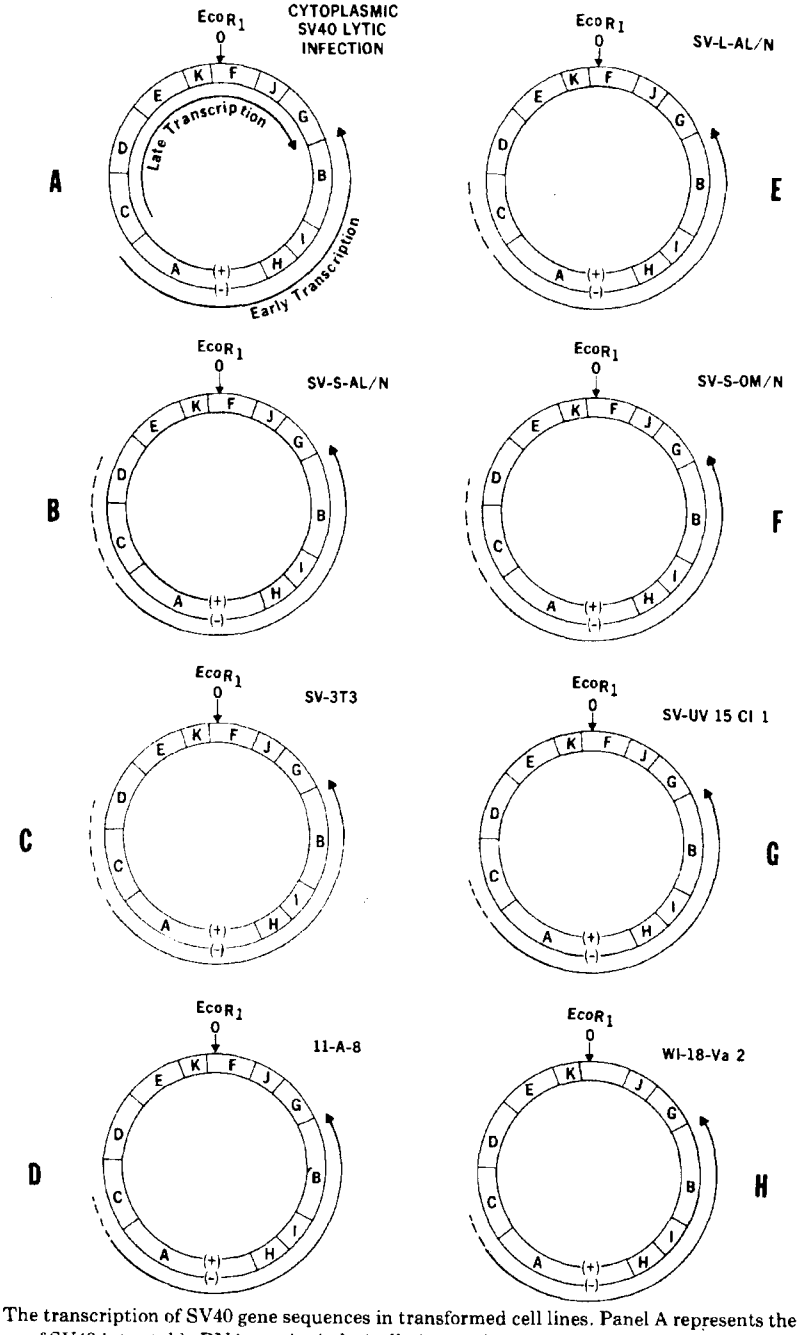

FIG. 3. The transcription of SV40 gene sequences in transformed cell lines. Panel A represents the pattern of transcription of SV40 into stable RNA species in lytically infected cells (Khoury *et al.*, 1973b and manuscript in preparation). In panels B–H are represented the abundant (——) and scarce (-----) viral RNA sequences in various SV40-transformed cell lines.

TABLE 3

EXTENT OF HYBRIDIZATION OF TRANSFORMED CELL RNA'S WITH THE MINUS STRANDS OF *Hin* FRAGMENTS A AND B

Source of RNA	RNA concentration (mg/ml)	Minus strand of *Hin* fragment (% hybrids)[a]	
		A	B
SV40 late lytic[b]	1.0	60	40
SV40 late lytic (cytoplasmic)	0.5	89	86
SV40 cRNA	1×10^{-3}	97	90
SV-S-AL/N	6.8	75	86
SV-3T3	2.1	86	81
WI-18-Va2	5.2	90	80
SV-S-OM/N	3.2	88	85
11-A-8	3.3	82	75

[a] Percent DNA in duplex molecules after hybridization was analyzed by S_1 nuclease as described in Materials and Methods.

[b] These results from Khoury *et al.* (1973b).

preparation). Thus these hybridization experiments confirm previous results indicating that the abundant virus-specific RNA species synthesized in transformed cells is early viral RNA. The hybridization results with the minus strands of fragments *Hin* C and *Hin* D suggest that at least some of the SV40-specific RNA molecules in the transformed cell RNA populations contain sequences not present in the abundant species of lytic RNA. This finding is consistent with the conclusion that regions of the early template strand that are adjacent to the "early" segment of SV40 DNA are transcribed in transformed cells.

DISCUSSION

Hybridization of total transformed cell RNA to the separated strands of SV40 DNA have suggested the existence of abundant and scarce species of SV40-specific RNA (Khoury *et al.*, 1973a). This observation is further supported by the findings in the present study. The abundant species of SV40-specific RNA in transformed cells reacts with the same *Hin* fragments of viral DNA as early lytic RNA and is very likely identical to early SV40 RNA. Similar results in the mapping of SV40-transformed cell RNA have been obtained by others (Ozanne, B. and Sambrook, J., personal communication). Since this segment of RNA comprises 45–50% of the genome, it could code for a protein of approximately 100,000 daltons. Present estimates for the molecular weight of T-antigen (70,000–100,000; Del Villano and Defendi, 1973; Tegtmeyer, personal communication) are therefore compatible with its being a viral coded protein.

In each of the transformed cell lines examined, a second species of viral-specific RNA, present at significantly lower concentrations, was found (Figs. 2 and 3). The scarce viral-specific RNA sequences were, for the most part, complementary to the minus DNA strand and, in each case, represented regions of fragment *Hin* C, and in some cell lines, of fragment *Hin* D. It should be noted that fragments *Hin* C and D are located at the 3′ end of the early template strand of DNA (Fig. 3). In some transformed lines RNA was present that reacted to a small extent with the minus strand of fragment *Hin* G. Such hybridization was always significantly lower than the reaction with the minus strands of fragments *Hin* C and D. Thus, the "anti-late" sequences previously described appear to comprise the less abundant class of RNA and are complementary to the portions of the minus DNA strand adjacent to and on the 3′-DNA side of the early region (Fig. 3). It should be noted that the studies were performed with total transformed cellular RNA. Whether a processing step similar to that observed in the lytic cycle results in the retention of these "anti-late" RNA species within the nucleus is presently under investigation.

At least two explanations for the observed pattern of transcription can be entertained (Fig. 4). Both rely on the observation that transcription on the minus strand proceeds counterclockwise on the cleavage map (Khoury *et al.*, 1973b, Sambrook *et al.*, 1973). In one model (A), the early viral promoter (P_E) and the terminator (T_E) of integrated viral DNA are recognized. Less frequently, transcription would begin at a site in the cellular DNA and continue into the covalently linked viral DNA. This latter concept is supported by the existence

of RNA molecules in transformed cells containing host cell and viral sequences (Wall and Darnell, 1971). Since the "anti-late" viral RNA reacts predominantly with sequences adjacent to the 3′ end of the "early region," it seems more than likely that transcription is initiated within the host DNA and continues along the minus viral DNA strand. As mentioned previously (Khoury *et al.*, 1973a) this model places a restriction on the integration of SV40 DNA in transformed cells; the minus strand of viral DNA must be covalently linked to the strand of host cell DNA that is transcribed by the cellular RNA polymerase. It should be pointed out that the very low levels of viral specific RNA that hybridize with the minus strand of fragment *Hin* G may arise when the early terminator (T_E) is not recognized. If this general model (Fig. 4A) is correct, it suggests that the site of recombination in the viral genome, when integrating into the host DNA, is in the late genes and, depending on the transformed cell line, within fragment *Hin* C or D.

An alternative explanation for the observed pattern of hybridization involves the transcription of an extensive portion of the minus strand of the integrated SV40 genome (Fig. 4B). Subsequent degradation of all but the early viral RNA would result in the relative abundance of these sequences. The scarce classes of RNA homologous to *Hin* fragments C and D might then reflect a gradient (in the 5′ to 3′ direction on the RNA) of incompletely degraded RNA species. Such processing would be least efficient for the RNA transcribed from those *Hin* fragments adjacent to the 5′-RNA end of the "early region." If this is the case, an analysis of SV40-transformed cellular RNA may not provide information indicating whether the viral genome is integrated at a specific site(s).

Although the relationship between integration and the mechanism of transcription is still unclear, it is interesting to note that low but detectable amounts of SV40 RNA complementary to the minus strand of *Hin* C and G are present in primary monkey kidney cells infected with E46+ population of Adeno-SV40 hybrid virions

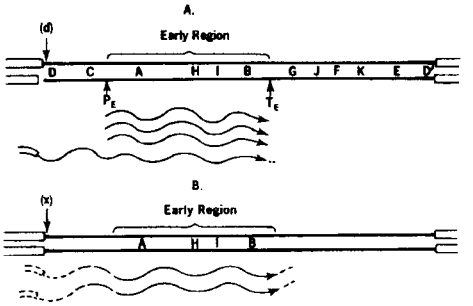

FIG. 4. Two models for the transcription of SV40 DNA in transformed cells lines. See the text for a description of each model. ⊐, host cell DNA; ⎯, viral DNA; P_E and T_E are the putative promoter and terminator for transcription of the early viral genes; ——, abundant viral RNA sequences; -----, scarce RNA sequences; A-K refer to *Hin* fragments of SV40 DNA; (d) represents a specific cleavage site (in *Hin* D) for integration of viral into host cell DNA; (x) represents a random cleavage site (in the late SV40 genes) for integration.

in addition to the more abundant "early" viral specific RNA. (Lebowitz and Khoury, submitted for publication). The E46+ genome contains a portion of SV40 DNA that includes segments of fragments *Hin* G and C in addition to the early SV40 gene region. The nondefective group of Adeno-SV40 hybrid viruses, Ad_2+ND 1–5, also contains portions of the SV40 genome integrated within adenoviral DNA. An analysis of the transcription of SV40 sequences from these virions likewise suggested the presence of a class of "anti-late" viral RNA complementary to the minus strand of fragment *Hin* G (Khoury *et al.*, 1973c).

Results from previous studies indicated that little or no RNA complementary to the plus (late) DNA strand could be found in most SV40 transformed cells by the hybridization methods employed. RNA was present in three of 12 transformed lines studied which hybridized to a small extent (4–7%) with the plus strand of SV40 DNA (Khoury *et al.*, 1973a, Martin and Khoury, 1973). When two of these RNA preparations were incubated with the plus strand of individual *Hin* fragments, homologous RNA sequences could not be localized to a specific region of the viral genome. This could reflect the fact that RNA species homologous to the plus SV40 DNA strand

are present at such low levels that they are either undetectable or anneal preferentially with the homologous (abundant) SV40-specific RNA. The factors regulating the expression of the plus strand of SV40 DNA in certain virus transformed cells are still unclear.

ACKNOWLEDGMENTS

This work was supported by grants from the National Institutes of Health and the Whitehall Foundation. We thank Peter Howley for helpful suggestions and Myles Brown for technical assistance.

REFERENCES

ALONI, Y., WINOCOUR, E., and SACHS, L. (1968). Characterization of simian virus 40-specific RNA in virus-yielding and transformed cells. *J. Mol. Biol.* **31,** 415–429.

ALONI, Y. (1972). Extensive symmetrical transcription of simian virus 40 DNA in virus-yielding cells. *Proc. Nat. Acad. Sci. USA* **69,** 2404–2409.

ANDO, T. (1966). A nuclease specific for heat-denatured DNA isolated from a product of Asperigillus oryzae. *Biochim. Biophys. Acta* **114,** 158–168.

DANNA, K., and NATHANS, D. (1971). Specific cleavage of simian virus 40 DNA by restriction endonuclease of Hemophilus influenzae. *Proc. Nat. Acad. Sci. USA* **68,** 2913–2917.

DANNA, K., and NATHANS, D. (1972). Bidirectional replication of simian virus 40 DNA. *Proc. Nat. Acad. Sci. USA* **69,** 3097–3100.

DANNA, K., SACK, G., and NATHANS, D. (1973). Studies of simian virus 40 DNA. VII. A cleavage map of the SV40 genome. *J. Mol. Biol.* **78,** 363–376.

DEL VILLANO, B. C., and DEFENDI, V. (1973). Characterization of SV40 T. antigen. *Virology* **51,** 34–46.

KHOURY, G., and MARTIN, M. A. (1972). A comparison of SV40 DNA transcription *in vivo* and *in vitro*. *Nature New Biol.* **238,** 4–6.

KHOURY, G., BYRNE, J. C., and MARTIN, M. A. (1972). Patterns of simian virus 40 DNA transcription after acute infection of permissive and nonpermissive cells. *Proc. Nat. Acad. Sci. USA* **69,** 1925–1928.

KHOURY, G., BYRNE, J. C., TAKEMOTO, K. K., and MARTIN, M. A. (1973a). Patterns of simian virus 40 DNA transcription. II. In transformed cells. *J. Virol.* **11,** 54–60.

KHOURY, G., MARTIN, M. A., LEE, T. N. H., DANNA, K., and NATHANS, D. (1973b). A map of simian virus 40 transcription sites expressed in productively infected cells. *J. Mol. Biol.* **78,** 377–389.

KHOURY, G., LEWIS, A. M., OXMAN, M. N., and LEVINE, A. S. (1973c). Strand orientation of SV40 transcription in cells infected by the nondefective adenovirus 2-SV40 hybrid viruses. *Nature New Biol.* **246,** 202–205.

KOHNE, D. (1969). In *Yearb. Carnegie Inst.* **67,** 310–320.

LINDSTROM, D. M., and DULBECCO, R. (1972). Strand orientation of SV40 transcription in productively infected cells. *Proc. Nat. Acad. Sci. USA* **69,** 1517–1520.

MARTIN, M. A., and KHOURY, G. (1973). Transcription of SV40 DNA in lytically infected and transformed cells. *In* "Virus Research," (C. F. Fox and W. S. Robinson, eds.), pp. 33–50. Academic Press, New York.

ODA, K., and DULBECCO, R. (1968). Regulation of transcription of SV40 DNA in productively infected and in transformed cells. *Proc. Nat. Acad. Sci. USA* **60,** 525–532.

OZANNE, B., SHARP, P. A., and SAMBROOK, J. (1973). Transcription of simian virus 40. II. Hybridization of RNA extracted from different lines of transformed cells to the separated strands of SV40 DNA. *J. Virol.* **12,** 90–98.

PATCH, C. T., LEWIS, A. M., and LEVINE, A S. (1974). Studies of nondefective adenovirus 2-SV40 hybrid viruses. IX. Template topography in the early region of SV40. *J. Virol.* **13,** 677–689.

PENMAN, S. (1966). RNA metabolism in the HeLa cell nucleus. *J. Mol. Biol.* **17,** 117–130.

SAMBROOK, J., SHARP, P. A., and KELLER, W. (1972). Transcription of simian virus 40. I. Separation of strands of SV40 DNA and hybridization of the separated strands to RNA extracted from lytically infected and transformed cells. *J. Mol. Biol.* **70,** 57–71.

SAMBROOK, J., SUGDEN, B., KELLER, W., and SHARP, P. A. (1973). Transcription of simian virus 40. III. Orientation of RNA synthesis and mapping of 'early' and 'late' species of viral RNA extracted from lytically infected cells. *Proc. Nat. Acad. Sci. USA* **70,** 3711–3715.

SAUER, G., and KIDWAI, J. (1968). The transcription of the SV40 genome in productively infected and transformed cells. *Proc. Nat. Acad. Sci. USA* **61,** 1256–1263.

SUTTON, W. D. (1971). A crude nuclease preparation suitable for use in DNA reassociation experiments. *Biochim. Biophys. Acta* **240,** 522–531.

WALL, R., and DARNELL, J. E. (1971). Presence of cell and virus specific sequences in the same molecules of nuclear RNA from virus transformed cells. *Nature New Biol.* **232,** 73–76.